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Comparison of sperm quality in different groups of rainbow trout spawners

by Yu.P. Babushkin, G.G. Savost'yanova and M.K. Chapskaya

The sperm from male rainbow trout is characterized here. We have established that the quality of the sperm depends on the age of the spawners. With an increase in age, we note a certain increase in the activity of the spermatozoa with a simulatancous decrease in their concentration. The same picture is observed when males of different age are compared within individual breeds. We have established a relationship between the volume of the ejaculate and the weight and age of the male trout. The males of the Danish group of trout display the highest activity of spermatozoa and the largest volume of ejaculate in comparison with the Czechoslovak and Gostilitsy trout. At the same time, the Danish group of males is inferior to the Czechoslovak and Gostilitsy males in the concentration of spermatozoa.

The sperm from 4-year-old males is characterized by a higher fertilizing capacity, as is the sperm of the Danish group of males in comparison with the other groups. Six tables and 25 bibliographies are included.

As we know, the quality of the progeny is greatly affected by the age of the spawners. [Scientists] began to study this question comparatively recently. F.G. Martyshev (1953, 1958, 1961), I.M. Anisimova (1956, 1961) and A.I. Kanayev (1955) examined how the spawning age affected the progeny in carp. However, they did not examine how the age of the male fish affected the quality of the sperm. Generally speaking, far less attention has been devoted to the sperm than to the egg when studying fish gametes. Meanwhile, experiments with mammals have shown that the quality of the males and their sperm has a considerable influence on the progeny (Milovanov, 1962).

The majority of the investigations devoted to the study of sperm from different species of fish characterizes the concentration and duration of the active state of the spermatozoa, sperm storage conditions and sperm transportation (Cherfas, 1940; Musselius, 1951; Pisarenkova, 1958; Smirnov, 1963). For example, V.A. Musselius gives the following

data on the concentration and activity of spermatozoa in different species of fish:

	Concentration, millions/mm^3	Activity, min
Carp	26-28	3
Crucian carp	18.7	1.0-2.3
Perch	35.6	1.0-2.3
Roach	11.1	2.0-4.0
Bream	19.1	-
Tench	4.9	11.5

According to B.I. Cherfas (1940), the active state of the spermatozoa lasts 45 seconds in the Atlantic salmon, and one minute in the brook trout.

In the past, fish breeders relied exclusively on practical experience when evaluating the age of the male fish and the quality of their sperm (Grimm, 1916; Yevtyukhin, 1933; Yeleonsky, 1936; Cherfas, 1956). A number of authors have demonstrated the importance of male qualities, including sperm quality, in the reproduction of fish (Persov, 1951; Nikol'sky, 1953; Sadov, 1957; Smirnov, 1963).

There are very few papers on the relationship between the quality of fish sperm and the age of the fish. This relationship in rainbow trout was noted by Scheuring (1924). He noted that the active state of the spermatozoa lasted the longest in middle-aged fish.

The most complete data have been obtained by V.N. Zhukinsky (1965) for Azov roach, and by F.G. Martyshev, I.M. Anisimova and Yu.A. Privezentsev (1967) for carp. These authors have established that there is absolutely no relationship between the age of the males, the concentration of the spermatozoa and the duration of the active state of the latter. They have also shown that the number of unviable spermatozoa changes with the growth of the males, and in Azov roach the volume of the ejaculate also increases sharply.

The purpose of our investigations was to study the influence of male age on sperm quality, and to compare the latter in males of different groups of rainbow trout.

Material and Method

In order to characterize the quality of the sperm in rainbow trout spawners, sperm samples were taken from 145 male specimens. The following characteristics of fresh sperm were studied: the concentration of spermatozoa, the number of unviable spermatozoa, the duration of the active state of the spermatozoa, and the volume of the ejaculate.

The duration of the active state of the spermatozoa was determined with the help of a stopwatch by the method described (Scheuring, 1924; Stroganov, 1938; Musselius, 1951). The activity of the spermatozoa was measured 3-5 times in a row in each male, and then the results were averaged.

The concentration of the spermatozoa was determined by the method used to count erythrocytes (Puchkov, 1954; Golodets, 1955). The sperm was diluted 200-fold with 2% NaCl in a mixer, and then a drop from the mixer was placed in a Goryayev chamber and the number of spermatozoa counted in 80 small squares. The sperm concentration (K) was calculated by the formula

$$K=\frac{n \times 200}{V},$$

where **n** denotes the number of spermatozoa in one small square; **200** is the dilution coefficient; and

$V=\frac{1}{4000}$ mm^3 is the volume of one small square.

The volume of the ejaculate was measured with the help of a graduated cylinder.

We attempted to determine the percentage of unviable spermatozoa by the method evolved by V.N. Zhukinsky (1965) and successfully used to study the sperm in Azov roach and carp. However, we could not use this method for rainbow trout. When the smears were prepared, we observed no difference in the colour of the live and dead spermatozoa.

Dependence of sperm quality on spawning age

As our investigations have shown, sperm quality in rainbow trout spawners depends on their age (Table 1).

Table 1. Sperm characteristics of male rainbow trout of different age

Age	No. of specimens studied	Weight, g	Length, cm	Fulton's condition factor	Activity of spermatozoa, s	Concentration of spermatozoa, mil/mm^3	Volume of ejaculate, cm^3
2	115	375	30.2	1.32	30.2	11.2	2.2
3	40	858	39.6	1.34	26.3	8.3	5.0
4	69	1594	49.3	1.29	36.3	7.4	8.9

As we can see from Table 1, the active state of the spermatozoa in rainbow trout is not long-lived. We observe a certain increase in the activity of the spermatozoa in 4-year-old males (an average 36.3 s), the duration of the active state of the spermatozoa varying from 16 to 56 s. At the same time, we note a decrease in the concentration of spermatozoa and an increase in the volume of the ejaculate as the fish age. The same picture was observed when we compared male specimens of different age within different breeds of rainbow trout (Table 2).

Table 2. Sperm characteristics of male rainbow trout of different age and breeds

Fish group	Age	No. of specimens studied	Weight, g	Length, cm	Fulton's condition factor	Activity of spermatozoa, s	Concentration of spermatozoa, mil/mm^3	Volume of ejaculate, cm^3
Danish	2	37	480	32.7	1.34	32.0	10.1	3.0
	3	10	1011	41.5	1.38	31.3	8.1	5.2
	4	33	1730	50.8	1.32	40.6	6.0	10.4
Czechoslovak	2	27	334	29.3	1.31	25.0	10.6	1.6
	3	10	935	41.5	1.27	25.6	7.1	6.6
	4	21	1319	46.7	1.28	34.2	8.3	6.1
Gostilitsky	2	25	305	28.7	1.28	31.0	12.1	2.5
	3	10	729	37.6	1.34	22.6	8.9	4.0
	4	15	1684	48.6	1.28	29.4	9.2	9.8

As we know, the size of the gonads also increases with the body size of the spawners. The fecundity of female rainbow trout increases with their weight (Savost'yanova, present collection). We assume that the fecundity of the males also increases with their growth. To determine the relationship between the volume of the ejaculate and the body size of the males, we measured the volume of a sperm specimen taken at the same time from 145 spawners, and have shown that the volume of the ejaculate increases with the growth of the males. Our data are quite consistent with those obtained for Azov roach by V.N. Zhukinsky (1965).

Comparative sperm characteristics of male rainbow trout from different groups

During 1966-1968, we studied the sperm from males of three groups of trout, the Danish, Czechoslovak and Gostilitsky groups, raised under the same conditions.

Our 1966 investigations showed that the activity of the spermatozoa and the volume of the ejaculate were somewhat lower in 2-year-old males of the Czechoslovak group of trout; practically no difference was observed in the rest of the characteristics (Table 3).

Table 3. Sperm characteristics of 2-year-old males from different groups of rainbow trout (1966)

Group of fish	No. of specimens studied	Weight, g	Length, cm	Fulton's condition factor	Activity of spermatozoa, s	Concentration of spermatozoa, mil/mm^3	Volume of ejaculate, cm^3
Danish	37	480	32.7	1.34	32.0	10.1	3.0
Czechoslovak	27	334	29.3	1.31	25.0	10.6	1.6
Gostilitsky	25	305	28.7	1.28	31.0	12.1	2.5
Average	-	386	30.5	1.31	28.0	10.8	2.4

In 1967, the same investigations were repeated with the males of the same groups, but one year older, i.e. three-year-olds (Table 4).

Table 4. Sperm characteristics of 3-year-old males from different groups of rainbow trout (1967)

Group of fish	No. of specimens studied	Weight, g	Length, cm	Fulton's condition factor	Activity of spermatozoa, s	Concentration of spermatozoa, mil/mm^3	Volume of ejaculate, cm^3
Danish	10	1011	41.5	1.38	31.3	8.1	5.2
Czechoslovak	10	935	41.5	1.27	25.6	7.1	6.6
Gostilitsky	10	729	37.6	1.34	22.6	8.9	4.0
Average	-	891	40.2	1.33	26.5	8.0	5.2

The highest activity of spermatozoa was observed in the Danish group of male trout. A particularly marked difference in this characteristic was noted between the Danish and the Gostilitsky trout. A certain decrease was noted in the length of the active state and concentration of the spermatozoa in the 3-year-old males as compared with the 2-year-olds.

The following year (1968), we studied the sperm of 4-year-old males from different groups of trout (Table 5).

Table 5. Sperm characteristics of 4-year-old males from different groups of rainbow trout (1968)

Group of fish	No. of specimens studied	Weight, g	Length, cm	Fulton's condition factor	Activity of spermatozoa, s	Concentration of spermatozoa, mil/mm^3	Volume of ejaculate, cm^3
Danish	33	1730	50.8	1.32	40.6	6.0	10.4
Czechoslovak	21	1319	46.7	1.28	34.2	8.3	6.1
Gostilitsky	15	1684	48.5	1.28	29.4	9.2	9.8
Average	-	1594	49.3	1.29	36.2	7.4	8.9

The highest sperm activity and volume of ejaculate were observed in the males of the Danish group of rainbow trout. However, the concentration of sperm was the lowest in the Danish group.

For a more detailed characterization of sperm quality, we attempted to determine the percentage of eggs fertilized by the sperm of males from different groups of rainbow trout. This was accomplished by

combining the eggs of several females and fertilizing them with the sperm from male fish of the Danish, Czechoslovak and Gostilitsky groups of rainbow trout. These experiments were carried out with 2-fold replication in 1966, and 3-fold replication in 1968 (Table 6). The percentage of fertilization was determined at the "eye stage" with 100 eggs examined from each batch.

Table 6. Fertilizing capacity of sperm from male fish of different groups of rainbow trout (%)

Group of fish	Two-year-olds (1966)			Four-years-olds (1968)			
	1st repl.	2nd repl.	aver-age	1st repl.	2nd repl.	3rd repl.	aver-age
Danish	70	75	72.5	94.3	97.1	98.1	96.4
Czechoslovak	66	61	63.5	98.3	83.4	90.9	91.2
Gostilitsky	65	53	59.0	98.2	91.9	90.1	92.8

The highest percentage of egg fertilization was noted when sperm from the Danish group was used. Male rainbow trout breed for the first time at the age of two years. At this age, the fertilizing capacity of the males is quite low (59.0-72.5%). The results of egg fertilization are much higher (91.2-96.4%) at the age of 4 years.

Unfortunately, we were unable to characterize the sperm quality of older male rainbow trout due to their unavailability. The first results of sperm quality testing have shown that it is better to use 4-year-old males for egg fertilization.

Summary

1. The sperm quality in rainbow trout spawners depends on the age of the fish. A certain increase is observed in the activity of the spermatozoa in 4-year-old male trout.

2. The volume of the ejaculate in male trout increases with age, while the concentration of the spermatozoa decreases somewhat.

3. The male trout of the Danish group are characterized by the highest activity of the spermatozoa and the highest volume of the ejaculate. At the same time, they are much farther behind the males of

the Czechoslovak and Gostilitsky groups in the concentration of the spermatozoa.

4. The male trout of the Danish group are superior to those of the other groups in fertilizing capacity.

5. Within each breed of rainbow trout, the 4-year-old males display the highest fertilizing capacity.

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